Regulatory Peptides 173 (2012) 47–54

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Neutral aminopeptidase and dipeptidyl peptidase IV in the development of collagen II-induced arthritis

Simone Cristina Yamasaki, Stephanie Murari-do-Nascimento, Paulo Flavio Silveira *

Laboratory of Pharmacology, Instituto Butantan, Av. Vital Brasil, 1500, 05503-900 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 5 May 2011
Received in revised form 11 August 2011
Accepted 16 September 2011
Available online 6 October 2011

Keywords:
Aminopeptidase
CD13
CD26
Collagen-induced arthritis
Rheumatoid arthritis

ABSTRACT

This study evaluated the hypothesis that neutral (APN) and dipeptidyl-IV (DPPIV) aminopeptidase activity levels would be critical for the susceptibility to arthritis in collagen-induced model (CIA). The macroscopic signs of arthritis in CIA rats were checked and peripheral blood, synovial fluid and synovial tissue from knee joint were withdrawn. Soluble (SF) and solubilized membrane-bound (MF) fractions from the synovial tissue and peripheral blood mononuclear cells (PBMCs) were obtained. APN and DPPIV activities were fluorometrically quantified. Severe swelling in both the entire hind paws was the minimum criterion to select CIA rats with arthritis. These arthritic rats had high APN in plasma, synovial fluid and SF of the synovial tissue, together with low APN and DPPIV in MF of PBMCs and hallmark histological changes in tibio-tarsal joint. CIA rats with no macroscopic signs of arthritis were diagnosed as resistant and they had low APN in MF of the synovial tissue, low DPPIV in SF of PBMCs and high DPPIV in plasma together with histological aspects of tibio-tarsal joint similar to healthy control rats. Data suggested that APN and DPPIV activity levels are related to the development of arthritis, being protective or inducer of the susceptibility. Understanding what is controlling the compartment-specific changes of these peptidases and looking at ways in which to manipulate their activities may lead to a better knowledge of the arthritic processes and novel treatments.

1. Introduction

Collagen-induced arthritis (CIA) is a T cell-dependent animal model of autoimmunity in which susceptibility has been associated with genes expressed at different stages of the disease within and outside the major histocompatibility complex (MHC), and CIA has been studied extensively because of its similarities to human rheumatoid arthritis (RA) [1–4]. While the direct role of T cells in the pathogenesis of CIA is unclear, the B cell immunoglobulin response to collagen type II (CII) is critical to the development of CIA, and also the transgenic expression of certain molecules associated with susceptibility to RA such as HLA-DR1 (*0101) or DR4 (*0401) is known to confer susceptibility to CIA [5]. Despite advances, additional unidentified molecular susceptibility factors must be associated with increased predisposition to develop RA. Standard diagnosis, prognosis and therapy for RA still have also numerous limitations. Heterogeneous cellular pathways and biochemical mechanisms are responsible for generating the glycoprotein complexes of peptide and MHC that are displayed on the surfaces of antigen-presenting cells for recognition by T lymphocytes [6]. Recently, the glycopeptide CII259–273, a fragment from CII, was shown to induce tolerance in mice susceptible to CIA, opening a new perspective to the development of an altered glycopeptide for inducing immunological tolerance in CIA and a possible way for a therapeutic vaccine for the treatment of RA [7]. Furthermore, changes in proteins implicated in matrix degradation, cell activation, inflammation and bone collagen degradation products accompany early knee and osteoarthritis development and can precede radiographic detection by several years [8].

Aminopeptidase (AP) enzymes are ubiquitous exopeptidases that catalyze the removal of one or more N-terminal residues from target proteins, peptides and amide or arylamide derivative of amino acids [9]. The functions of these enzymes in RA are controversial and poorly understood, though they have been shown to participate in the cleavage of antigenic peptides in endoplasmic reticulum, and in so doing either create or destroy MHC class I-presented epitopes [10], as well as in mediator generation and associated inflammation [11–17]. Recently, basic AP (APB) activity was correlated with susceptibility to develop edema and high levels of serum TNF-alpha in a rat CIA model [18]. Among other AP enzymatic activities that have been also consistently associated with inflammatory and immune processes the most outstanding are neutral AP (APN) and dipeptidyl peptidase IV (DPPIV) [11–17,19–20].

Abbreviations: Ang, angiotensin; AP, aminopeptidase; APN, neutral aminopeptidase; BSA, bovine serum albumin; CII, type II collagen; CIA, collagen-induced arthritis; DPPIV, dipeptidyl peptidase IV; HE, hematoxylin–eosin; LDH, lactate dehydrogenase; MF, membrane-bound fraction; NADH, nicotinamide adenine dinucleotide, reduced form; NFκB, nuclear factor-kappa B; PBMCs, peripheral blood mononuclear cells; PGP, Pro-Gly-Pro; RA, rheumatoid arthritis; SF, soluble fraction.

* Corresponding author. Tel.: +55 11 7067-9058; fax: +55 11 3726 7222 (2061).
E-mail address: pefesil@butantan.gov.br (P.F. Silveira).

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doi:10.1016/j.regpep.2011.09.004

To test the hypothesis that susceptibility and development of RA is characterized by altered activity levels of APN and DPPIV, they were quantified in the synovial fluid, blood plasma and soluble (SF) and solubilized membrane-bound (MF) fractions from the synovial tissue of the knee and from peripheral blood mononuclear cells (PBMCs) of control and CII-treated rats which developed or not arthritis.

2. Materials and methods

2.1. Animals and treatments

Sixty-six adult male Wistar rats, weighing 150–160 g, were maintained in polyethylene cages (inside length × width × height = 56 × 35 × 19 cm) with food and tap water ad libitum, in a container with controlled temperature (25 °C), relative humidity (65.3 ± 0.9%) and 12 h:12 h photoperiod light:dark (lights on at 6:00 am). The animals and research protocols used in this study are in agreement with the COBEA (Brazilian College of Animal Experimentation) and were approved by the Ethics Committee of the Instituto Butantan (432/07). The animals were anesthetized with a solution of ketamine (Syntec, Brazil) (3.75%) and xylazine (Calmiun) (Agener Union, Brazil) (0.5%) at a dose of 0.2 mL/100 g body weight, via intraperitoneal, and then subjected to induction of arthritis as previously described by Mendes et al. [18]. Briefly, CII from chicken (Sigma, USA) dissolved in 0.01 M acetic acid and emulsified in an equal volume of Freund's incomplete adjuvant (Sigma, USA) (prepared at 4 °C just before use), was administered via a single intradermal dose of 0.4 mg/0.2 mL/animal, into the proximal one-third of the tail (induced animals, n = 50), or with 0.9% saline at the same scheme of administration (sham induction, n = 16). On day 41 after induction, significant changes in redness and/or swelling were macroscopically undetected in 30% or evident in 70% of rats. The rats with no macroscopic signs of arthritis were grouped as resistant to induction of CIA (n = 15). Severe swelling in both the entire hind paws was observed in 60% of rats, which were grouped as arthritic animals (n = 30). All the rats in intermediate level of swelling (10%) were not included in the present study. At this same day, after anesthesia using the same scheme specified above, the hind paw swelling was quantified by macroscopic measurement of the dorsal-plantar thickness in the region of the metatarsus with a micrometer (Mitutoyo, USA). Both paws were measured and calculated the average thickness of each animal. Joint pathology was examined by histology.

2.2. Obtaining samples

The blood was withdrawn from the left ventricle with heparinized syringes and centrifuged at 200 × *g* for 10 min at room temperature to separate plasma (supernatant) from the pellet containing PBMCs and other blood cells. As previously described by Mendes et al. [18], synovial fluid and synovial tissue were subsequently removed from both knees of each animal. The withdrawal of synovial fluid and synovial tissue was performed as follows: joint cavity was opened, the patellar ligament was carefully removed and then 200 μL of 0.9% NaCl was injected intraarticularly into each knee. Subsequently, the synovial fluid was aspirated with the same syringe. After this aspiration, the synovial membrane was excised together with connective tissue and infrapatellar fat pad of the joint capsule, after careful removal of the surrounding patellar adipose tissue.

2.3. Histology

Histological analysis was performed based on the methodology described by Hashmi et al. [21]. Briefly, hind right paws were fixed in 10% formalin for a week and transferred to 70% ethanol. Decalcification was performed in 5.5% EDTA solution for 60 days. The EDTA solution was changed every 48 h. After decalcification, the region of the tibio-tarsal joint was separated from the rest of the paw in order to be submitted to the preparation of histological slides. Dehydration was performed with increasing concentrations of ethanol (70%, 95%, 95%, 100%, 100% and 100%). Then, ethanol was replaced by xylene in three consecutive baths. In turn, the xylene was replaced with paraffin at 60 °C in three consecutive baths. Then, the samples were embedded in paraffin melted. The cuts were made longitudinally in the thickness of 5 μm, for mounting the slides. The slides were stained with hematoxylin–eosin (HE). The analysis was performed under a light microscope Nikon E600 equipped with a digital camera Cool-SNAP-PRO®, using the Image-Pro Plus® 4.0 software (Cybernetics).

2.4. Separation and counting of PBMCs

It was performed according to the methodology adapted from Böyum [22] and Tamura et al. [23] as follows. The pellet resulting from the obtainment of blood plasma was reconstituted to the original volume with 0.9% NaCl and then carefully layered on an equal volume of Percoll (ρ = 1.077 mg/mL) (GE-Healthcare, USA) and subsequently centrifuged (200 × *g* for 20 min at room temperature). The layer containing the PBMCs was then removed from the tube and washed twice with 0.9% NaCl (1:3 v/v diluted blood cells) and centrifuged again at the same conditions to discard the supernatant. PBMCs were resuspended in 0.5 mL of 0.9% NaCl and the total number of PBMCs was assessed in 20 μL aliquots of this suspension diluted with Turk's fluid (1:20, v/v). Cell counting was performed in a Neubauer chamber under optical microscopy.

2.5. Fractionation of the synovial tissue and PBMCs

As previously described by Mendes et al. [18], the synovial tissue from both knees of each animal was homogenized in 10 mM Tris–HCl buffer, pH 7.4 (0.1 g tissue/3.0 mL) for 3 min at 15,000 rpm (homogenizer Polytron-Aggregate, Kinematica, Switzerland). PBMCs, resuspended in 0.9% NaCl, were sonicated in 10 mM Tris–HCl, pH 7.4 (3.0×10^6 cells/mL), for 10 s at amplitude level of 40 μm at a constant frequency of 20 kHz. These samples were then ultracentrifuged at 100,000 × *g* for 35 min (ultracentrifuge Hitachi CP60E). The resulting supernatants correspond to SF. The resulting pellets were washed twice with the same buffer and ultracentrifuged at 100,000 × *g* for 35 min, to assure the complet removal of SF. The pellet was homogenized for 3 min at 800 rpm (homogenizer Tecnal TE 099) with the same volume of the same buffer plus Triton X-100 (0.1%) and ultracentrifuged again (100,000 × *g* for 35 min). The resulting supernatants correspond to MF. All procedures were carried out at 4 °C.

As a marker for the synovial tissue and PBMCs fractionation procedure, lactate dehydrogenase (LDH) activity was determined, photometrically, at 340 nm, in triplicates of individual samples of SF and MF, as previously described [24]. LDH activity was obtained by subtracting the values of the second from that of the first reading, and extrapolated by comparison with a standard curve of NADH.

2.6. Total protein

Total protein was measured photometrically (Power Wave XR spectrophotometer, Bio-Tek, USA), at 630 nm, in triplicates of individual samples of 40 μL of synovial fluid, plasma (diluted 500-fold) and SF (diluted 10-fold) and MF (diluted 2-fold) from the synovial tissue and PBMCs, by the method of Bradford [25], using a Bio-Rad protein assay reagent (Hercules, USA). Protein contents were extrapolated by comparison with standard curves of bovine serum albumin (BSA) in the same diluent.

2.7. AP activities

AP activities were measured on the basis of the amount of β-naphthylamine (Sigma, USA) (for APN) [24] or 4-methoxy-β-naphthylamine (Sigma, USA) (for DPPIV) [26] released as a result of the enzyme activity in triplicates of individual samples incubated at 37 °C, for 30 min, in 96-well flat bottom microplates (Corning Inc., USA) with prewarmed substrate solution diluted to 0.125 mM for APN or 0.2 mM for DPPIV in respective 0.05 M buffers containing 0.1 mg BSA/mL, in a final volume of 300 μL. For APN activity 25 μL blood plasma (200 to 450 μg protein), 10 μL synovial fluid (5 to 20 μg protein), 10 μL SF (1.3 to 7.7 μg protein) and 25 μL MF (2.6 to 15 μg protein) from the synovial tissue, and 50 μL SF (22 to 72 μg protein) and 100 μL MF (13 to 44 μg protein) from PBMCs were incubated with L-alanine-β-naphthylamide (Sigma, USA) in phosphate buffer, pH 7.4, with 1 mM DL-dithiothreitol (Sigma, USA) (Sigma, USA). For DPPIV activity 25 μL blood plasma (51 to 112 μg protein), 10 μL synovial fluid (5 to 20 μg protein), 25 μL SF (2.8 to 20 μg protein) and MF (2.6 to 15 μg protein) from the synovial tissue, and 50 μL SF (22 to 72 μg protein) and 100 μL MF (13 to 44 μg protein) from PBMCs were incubated with H-Gly-Pro-4-methoxy-β-naphthylamide (Peninsula, USA) in Tris/HCl buffer, pH 8.3. β-Naphthylamine or methoxy-β-naphthylamine was estimated fluorometrically (FL600FA Microplate Fluorescence/Absorbance Reader Bio-Tek, USA), at 460/40 nm emission wavelength and 360/40 nm excitation wavelength. The value of incubates at zero time (blank) was subtracted and the relative fluorescence was converted to picomoles of β-naphthylamine or methoxy-β-naphthylamine by comparison with a correspondent standard curve. Enzyme activities were expressed as picomoles of hydrolysed substrate min^{-1} mg $protein^{-1}$.

2.8. Statistical analysis

Data are shown as mean ± standard error of the mean (SEM) and were analyzed statistically using the GraphPad Instat™ software package. Regression analyses were performed to obtain standard curves of protein, NADH, β-naphthylamine and methoxy-β-naphthylamine. Student's t-test was performed to compare values of LDH between SF and MF. One-way analysis of variance (ANOVA) followed, when differences were detected, by the Tukey–Kramer multiple comparison test was performed to compare values among control, arthritic and resistant groups. In all the calculations a minimum critical level of $p<0.05$ was set.

3. Results

Fig. 1 shows that LDH activity in SF was higher than in MF of synovial tissue in control and CIA animals with arthritis. The same pattern was observed in resistant CIA animals. Qualitatively similar results were obtained in SF and MF from PBMCs of all groups (data not shown).

All selected arthritic animals had dorsal-plantar thickness >7 mm in the region of the metatarsus of both hind paws, and all selected resistant animals had this thickness similar to control. There is no evidence of histological changes of articular cartilage and subchondral bone in control (Fig. 2I) and resistant (Fig. 2III) animal groups. However, in arthritic group (Fig. 2II) there is erosion in the cartilage and bone tissues, presence of pannus into intraarticular cavity and infiltration of cells.

Fig. 3 shows that APN activity is highly expressed in MF from the synovial tissue of all animal groups under study. In SF from the synovial tissue, APN activity was higher in arthritic than in control or resistant (Fig. 3A). Resistant and control animals had similar APN activity in SF from the synovial tissue (Fig. 3A). In MF from the synovial tissue APN activity was lower in resistant than in control or arthritic (Fig. 3B). Control and arthritic animals had similar APN activity in MF from the synovial tissue (Fig. 3B). Arthritic had higher APN activity than control or resistant animals in synovial fluid (Fig. 3C) and blood plasma (Fig. 3D). Resistant and control animals had similar APN activity in synovial fluid (Fig. 3C) and blood plasma (Fig. 3D).

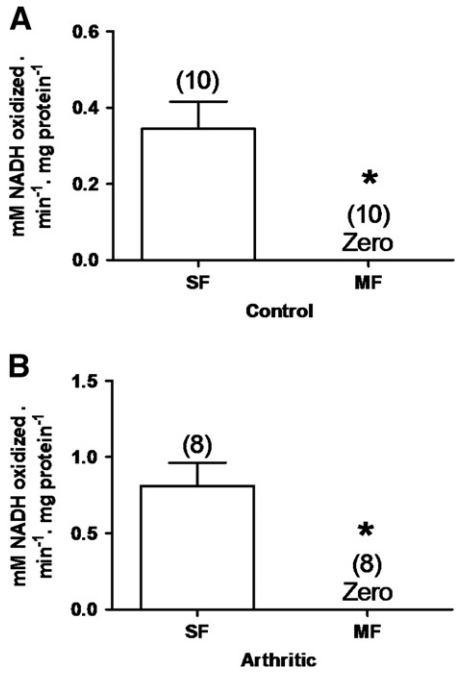

Fig. 1. Lactate dehydrogenase (LDH) activity in soluble fraction (SF) and solubilized membrane-bound fraction (MF) from synovial tissue of sham induced (control) (A) and CII-treated that developed hind paw edema (arthritic) (B). Values are means ± SEM. Number of animals in parentheses over the bars. *$p<0.0001$, unpaired two-tailed Student's t test.

Fig. 4 shows that APN activity is highly expressed in SF from PBMCs of all groups under study. APN activity in all groups under study was similar in SF from PBMCs (Fig. 4A). However, arthritic had lower APN activity than control or resistant animals in MF from PBMCs (Fig. 4B). Resistant and control animals had similar APN activity in MF from PBMCs (Fig. 4B).

DPPIV activity in all animal groups under study was similar in blood plasma (Fig. 5D) and SF (Fig. 5A) and MF (Fig. 5B) from synovial tissue, being relatively expressed at lower levels in synovial fluid (Fig. 5C). Arthritic, resistant and control animals had similar DPPIV activity in synovial fluid (Fig. 5C) and SF (Fig. 5A) and MF (Fig. 5B) of synovial tissue. In blood plasma, DPPIV activity was higher in resistant than in control or arthritic (Fig. 5D). Control and arthritic animals had similar DPPIV activity in blood plasma (Fig. 5D).

Fig. 6 shows that DPPIV activity is highly expressed in MF from PBMCs of all groups under study. Resistant animals had similar DPPIV activity than control, but lower than arthritic animals in SF of PBMCs (Fig. 6A), while arthritic had lower DPPIV activity than control or resistant in MF from PBMCs (Fig. 6B). Resistant and control animals had similar DPPIV activity in MF from PBMCs (Fig. 6B).

4. Discussion

As preconized by Mendes et al. [18], arthritis and resistance in CIA model can be initially evidenced macroscopically according to the presence of severe swelling in both the entire hind paws (arthritic), and total absence of changes in redness and/or swelling (resistant), both conditions which were confirmed here by correspondent typical histological aspects of the tibio-tarsal joint. Based on the macroscopic grading scale proposed by Erlandsson Harris et al. [27], all the arthritic animals selected in the present study had the maximum score.

LDH activity is well known as a cytosolic marker [9, 13–15, 18, 24, 27, 28] and its higher activity in SF in comparison with MF from the

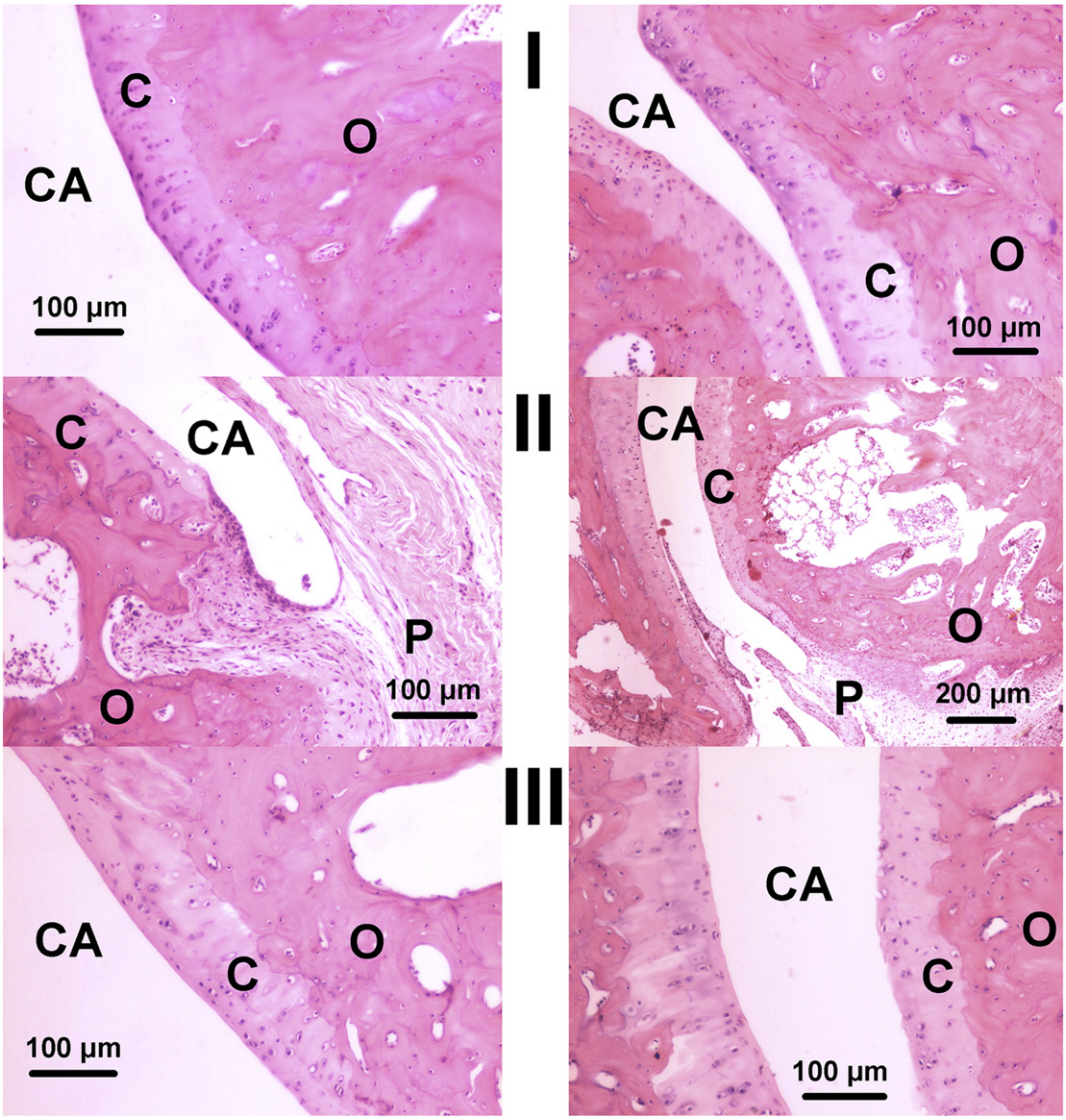

Fig. 2. Histology of the tibio-tarsal joint in longitudinal section. I (control animals) and III (animals resistant to induction of arthritis): the cartilage (C) and bone (O) are presented without histopathological changes and no cellular infiltration exists into the intraarticular cavity (CA). II (arthritic animals): the synovium is hyperplastic with pannus (P), invasion of CA and erosion of C and O. HE.

synovial tissue and PBMCs reflects the efficiency of the fractionation procedure. Thus, the present study is the first one to analyze APN and DPPIV activities in these separate fractions from the synovial tissue and PBMCs.

The hydrolytic ability of murine macrophages on six different naphthylamide derivative substrates for AP enzymes revealed a rise in activity levels of APN and DPPIV when these cells are elicited by thioglycolate [13]. The effects of modifying antirheumatic drugs, such as methotrexate and cyclosporine [19] have also been associated to altered levels of APN and DPPIV activities [14–15]. APN and DPPIV enzymatic activities are thought to be exhibited by various isozymes, including soluble forms in serum or plasma [29]. Little systematic data was available on the role of APN and DPPIV enzymatic activities in RA and none related to predisposition to develop RA. The present study shows that APN and DPPIV activities are expressed in synovial fluid, plasma and SF and MF from synovial tissue and PBMCs of control, arthritic and resistant animals. The present study mainly shows that resistant animals have higher DPPIV activity in plasma and lower DPPIV activity in SF from PBMCs together with lower APN activity in MF from the synovial tissue when compared with control or arthritic animals. In addition, APN activity is higher in the synovial fluid, plasma and SF from synovial tissue and lower in MF from PBMCs of arthritic animals when compared with control or resistant.

APN has been reported as an activation-associated molecule of T lymphocytes from the synovial fluid and the peripheral blood from patients suffering from RA or juvenile idiopathic arthritis [11]. In this sense, APN was supposed to participate in the mechanism of lymphocyte involvement in inflamed joints of RA patients as a lymphocyte chemoattractant [12]. In acute neutrophil driven inflammation, the neutrophil chemoattractant Pro-Gly-Pro (PGP), a biomarker for chronic obstructive pulmonary disease, is degraded by AP activity of leukotriene A4-hydrolase, which facilitates the resolution of inflammation, but in chronic situation the inhibition of this AP activity could lead to the accumulation of the chemoattractant and neutrophils [17]. APN is also being related to extracellular matrix degradation, antigen processing [30], T cell chemotaxis [12], motility and cellular adhesion during angiogenesis [31] and metabolism of enkephalins [32], substance P [33] and angiotensin (Ang) III [34]. The antinociceptive effect of enkephalins is well known [32] and substance P, a regulatory peptide, has effects on inflammation, modulating vascular tone and permeability, immune cell function and cellular proliferation [33]. Moreover, the hydrolyses of Ang III by APN and APB

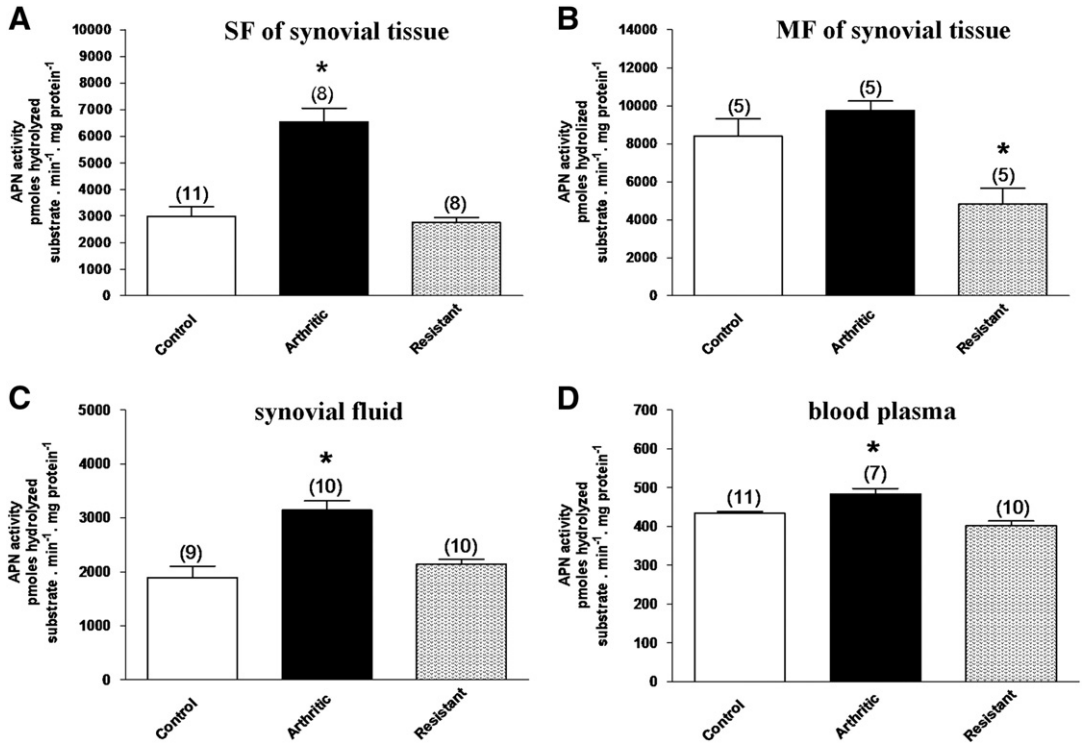

Fig. 3. APN activity of soluble (SF) (A) and solubilized membrane-bound (MF) (B) fractions from synovial tissue, synovial fluid (C) and blood plasma (D) in sham induced (control), CII-treated that developed arthritis and CII-treated rats that did not develop arthritis (resistant). Values are means ± SEM. Number of animals in parentheses over the bars. Analysis of variance ANOVA (A, $p<0.0001$; B, $p<0.002$; C and D, $p<0.0001$) with post-hoc Tukey–Kramer multiple comparisons test; *$p<0.05$ vs other two groups in the same panel.

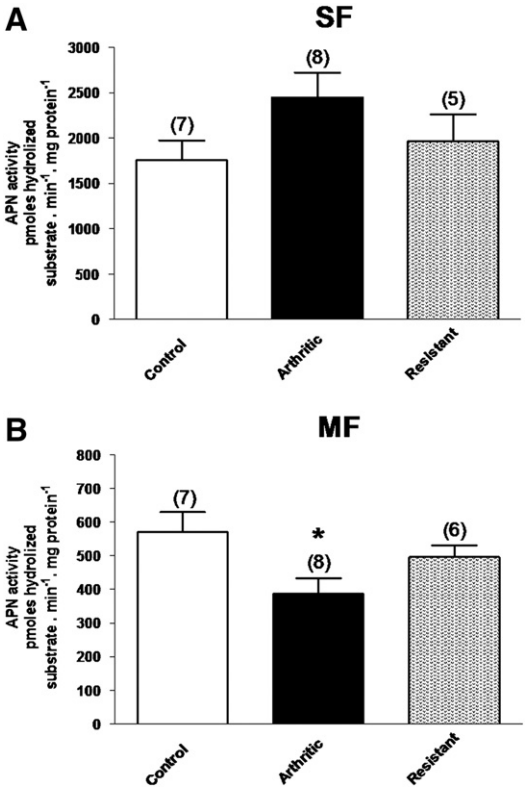

Fig. 4. APN activity of soluble (SF) (A) and solubilized membrane-bound (MF) (B) fractions from PBMCs in sham induced (control), CII-treated that developed arthritis and CII-treated rats that did not develop arthritis (resistant). Values are means ± SEM. Number of animals in parentheses over the bars. Analysis of variance ANOVA (A, $p=0.1610$; B, $p<0.044$) with post-hoc Tukey–Kramer multiple comparisons test; *$p<0.05$ vs other two groups in the same panel.

generate Ang IV, which activates the nuclear factor-kappa B (NFκB) pathway [34]. In the present study, higher APN activity in the synovial fluid, plasma and SF from the synovial tissue, as well as lower APN activity in MF from PBMCs of arthritic animals reinforce the hypothesis that, in addition to APB activity [18], changes on APN activity are related to pro- [35] and anti-inflammatory [36] roles of different Ang metabolites. Functionally, in the synovial fluid, the secreted form of APN could hydrolyze peptides such as Ang III and substance P and stimulate the maintenance of inflammation in the joint. In the cytosol, the increase of APN activity could also contribute to the proteolysis for the processing of MHC I ligands [37–38]. It is known that cytosolic peptides may bind to MHC II molecules for presentation to CD4+ cells [39]. Immunohistochemical studies have shown an increased APN in cellular membrane of synovial fibroblasts [12] and in the cellular membrane of synovial vessels [33] of patients with RA. Also by immunohistochemistry, it was observed an increase of APN/CD13 in synoviocytes [40] and in synovial tissue [41] of patients with RA. However, the detection of APN by immunohistochemistry is not enough to demonstrate its enzymatic activity [42]. In the present study, it was observed a decrease of APN activity in MF from the synovial tissue of animals resistant to CIA and from PBMCs of arthritic animals. This data suggests that the mechanism involved in reducing APN activity in the resistance to arthritis in CIA model might be opposite of the mechanism related to the increase of cytosolic APN in the synovial tissue and in MF from PBMCs, which characterize the development of the disease together with the decrease of DPPIV in MF from PBMCs. Probably, the mechanism could be related to APN-mediated plasma membrane instability, altering intracellular functions of this enzyme. The decrease of APN activity in MF from synovial tissue and the increase of DPPIV activity in plasma and the decrease of DPPIV in SF from PBMCs relative to control subjects can be an attractive tool for estimation of the susceptibility to develop RA. Therefore, based on current data and on the results presented here, it is probable that APN activity participates in peptide processing and chemotaxis

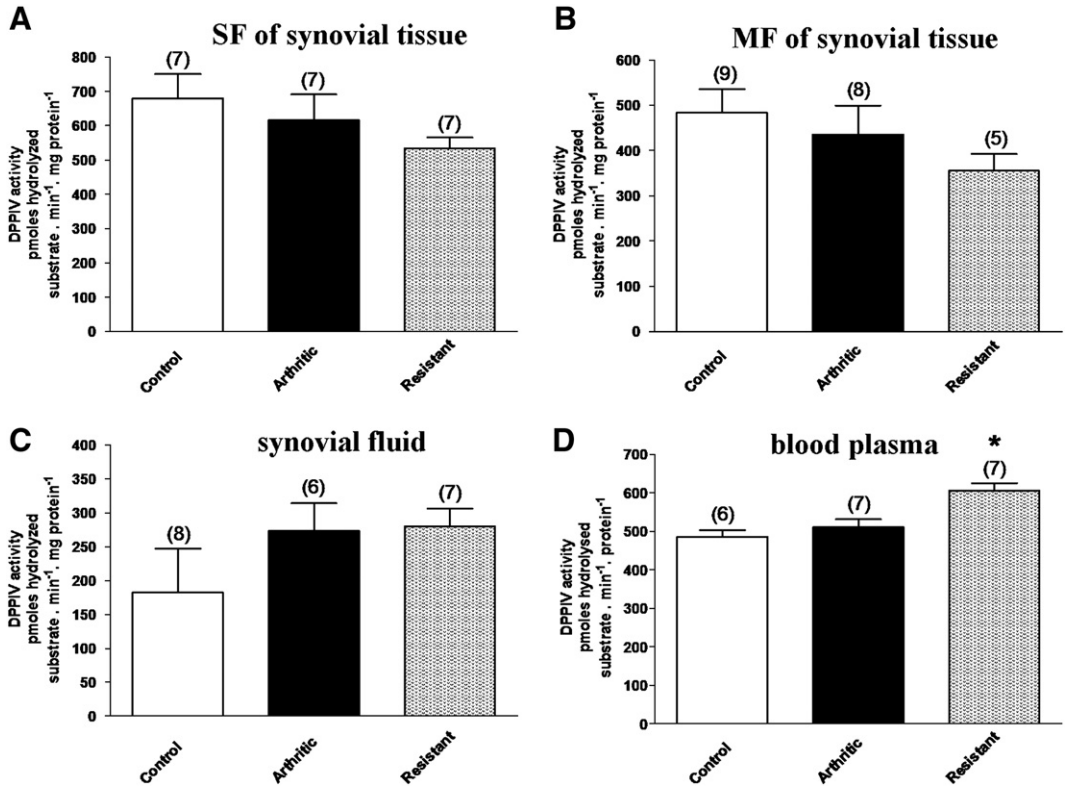

Fig. 5. DPPIV activity of soluble (SF) (A) and solubilized membrane-bound (MF) (B) fractions from synovial tissue, synovial fluid (C) and blood plasma (D) in sham induced (control), CII-treated that developed arthritis and CII-treated rats that did not develop arthritis (resistant). Values are means ± SEM. Number of animals in parentheses over the bars. Analysis of variance ANOVA (A, $p = 0.2808$; B, $p = 0.3504$; C, $p = 0.3039$; D, $p < 0.0014$) with post-hoc Tukey–Kramer multiple comparisons test; *$p < 0.05$ vs other two groups in the same panel.

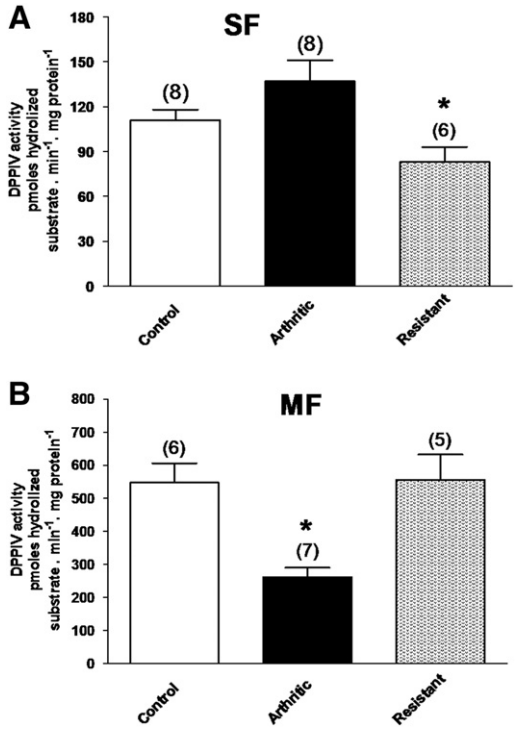

Fig. 6. DPPIV activity of soluble (SF) (A) and solubilized membrane-bound (MF) (B) fractions from PBMCs in sham induced (control), CII-treated that developed arthritis and CII-treated rats that did not develop arthritis (resistant). Values are means ± SEM. Number of animals in parentheses over the bars. Analysis of variance ANOVA (A, $p < 0.012$; B, $p < 0.0015$) with post-hoc Tukey–Kramer multiple comparisons test; *$p < 0.05$ vs arthritic in panel A; *$p < 0.05$ vs other two groups in panel B.

as a cytosolic factor related to RA establishment and a cellular membrane-bound factor related to the resistance to RA development.

DPPIV is known to be involved in the catabolism of chemokines, cytokines, substance P and other peptides [33, 43]. Moreover, DPPIV modulates lymphocyte immune responses [44]. Current data about DPPIV activity in RA are widely divergent. As reported by Sedo et al. [43], there is a significant decrease of DPPIV activity in synovial fluid in RA patients compared to healthy and osteoarthritic patients, which occurs together with an increased expression of DPPIV in membrane and decreased activity in plasma. According to Busso et al. [44], DPPIV in plasma is also decreased in arthritic mice. However, Suh et al. [45] reported no differences of DPPIV activity in plasma between control and arthritic animals. Mantle et al. [46] also described no alterations of DPPIV activity in plasma between healthy and RA patients. It has been proposed that DPPIV may have a dual role on RA and levels of DPPIV are associated with severity of RA [44]. Soluble DPPIV could inhibit cytokine production and membrane-bound DPPIV could stimulate cellular response in RA [44]. Low levels of DPPIV are related to RA severity [44], but on the other hand DPPIV inhibitors reduce the progression of arthritis in animal models [47]. In the present study, DPPIV activity was altered in plasma and SF from PBMCs of animals resistant to CIA, as well as in MF from PBMCs of arthritic animals. The increase of DPPIV in plasma and its decrease in MF from PBMCs could be related to the cleavage of circulating chemokines and cytokines, which respectively would prevent (resistant) or aggravate (arthritic) the inflammatory response of RA development. The decrease found in SF from PBMCs could be related to the prevalence of resting state of these cells in resistant animals. Different proportions of T/B cells and monocytes in PBMCs of arthritic, resistant and normal rats may also account for some of the differences seen in APN and DPPIV activities, mainly those in DPPIV, since it is believed that T cells are the major source of circulating DPPIV [48, 49]. However, the origin of DPPIV in plasma is not completely understood [48].

The mechanism of shedding DPPIV from the cell surface to the plasma is thought to be caused by circulating enzymes [50]. DPPIV is known to be present in the cellular membrane of different cell types, including papillary renal cell carcinoma [51]. In resting state T and NK cell present low expression of DPPIV, which is rapidly upregulated upon activation of these cells [49]. About 90% of circulating DPPIV activity is shed from transmembrane CD26/DPPIV [49, 50] and the remaining 10% are related with DPPIV-like activity, such as DPPII and fibroblast activating protein (FAP)-α [49]. Therapeutic potential of DPPIV inhibitors has been suggested for the treatment of RA [20] and the concomitant inhibition of both APN and DPPIV enzyme families by means of two separate inhibitors or by binary inhibitors with specificity for both enzyme families was demonstrated that synergistically affects immune cells on the level of cell cycle regulation, suppression of certain cytokines as well as the activation of regulatory T-cells [16]. Hence, the concept of a combined targeting of both families of peptidases for the treatment of inflammatory diseases has been preconized as a promising strategy [16].

5. Concluding remarks

APN and DPPIV activities are expressed in the synovial fluid, plasma and SF and MF from the synovial tissue and PBMCs of rats. Rats resistant to CIA are characterized by high DPPIV activity in plasma and low in soluble fraction from PBMCs together with low APN activity in MF from the synovial tissue. On the other hand, high APN in plasma, synovial fluid and SF of the synovial tissue, together with low APN and DPPIV in MF of PBMCs characterize the susceptibility to develop arthritis. Compartmentalized changes on APN and DPPIV activities represent potentially valuable new targets that may lead to a better understanding of the arthritic processes and novel treatments.

Acknowledgments

This investigation was financially supported by a Research Grant 07/08311-4 from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil). P.F.S was recipient of a Productivity Grant 301417/2008-3 from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil). S.C.Y. was recipient of a FAPESP fellowship. S.M-N was recipient of a CNPq fellowship. We thank all the staff at Laboratory of Pharmacology for technical support.

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